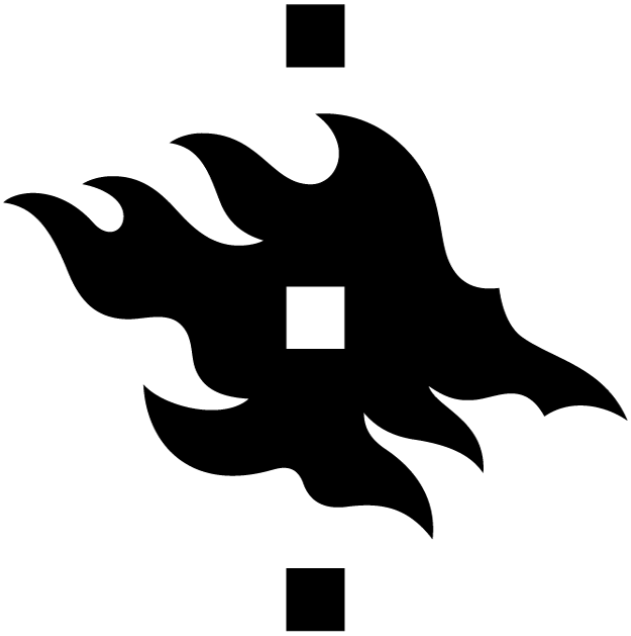
ISSN 0355-1180

UNIVERSITY OF HELSINKI

Faculty of Agriculture and Forestry

Department of Food and Nutrition



EKT Series 1905

House cricket (*Acheta Domesticus*) processing for food applications – focusing on drying and milling

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Helsinki 2019

Tiedekunta – Fakultet – Faculty	Koulutusohjelma – Utbildningsprogram – Degree Programme
Faculty of Agriculture and Forestry	Master's programme in Food Sciences

Tekijä – Författare – Author
Joonas Kauppinen

Työn nimi – Arbetets titel – Title
House cricket (*Acheta Domesticus*) processing for food applications – focusing on drying and milling

Oppiaine/Opintosuunta – Läroämne/Studieinriktning – Subject/Study track
FOOD-600 Food Technology study module

Työn laji – Arbetets art – Level	Aika – Datum – Month and year	Sivumäärä – Sidoantal – Number of pages
Master's Thesis	October 2019	42

Tiivistelmä – Referat – Abstract

Edible insects, such as house crickets (*Acheta domesticus*) are environmentally friendly, nutritious and safe alternative to meat, when special details such as allergenic potential and antinutrients are considered. The goal of this Master's thesis is to study the processing methods and parameters of house cricket in creating flour for food applications that is safe and of optimal quality. The thesis hopes to provide a reference for parameters used in house cricket drying and milling, with the equipment available. Furthermore, the goal is to study the optimal time to get below critical value in water content of 10 % and within critical values in water activity of 0,25-0,45 with an oven and a freeze-dryer. The samples within the critical safety values with minimal drying time are then milled with centrifugal mill with two different sieve-sizes and particle size distribution is measured and compared between oven-drying and freeze-drying, the two sieve-sizes and to reference flours. Particle size range of 45-150 μm was used as a goal. Optimal measured drying time at T=70 °C for oven-drying was 5 hours. For Freeze-drying at Tp=25 °C, Tc=-87 °C, p=1 mbar was 6 hours. Of the optimally dried samples, Oven-dried sample milled with 0,5 mm sieve size had the lowest mean and median particle size followed by Freeze-dried sample milled with 0,5 mm sieve size. The 5-hour Oven-dried sample milled with 0,5 mm sieve size was thus the closest to the optimal product, because it had the smallest particle size from all the samples with minimal drying time. Samples milled with 1 mm sieve size had coarser mean and median particle size similar to that of the reference samples obtained from the industry. Furthermore, it can be concluded that compared to reference flours, finer house cricket flour can be milled with 0,5 mm sieve size. On the other hand, 1 mm sieve size with the particular centrifugal mill used yields a flour with similar coarse particle size than those used in the industry today. However, Oven-dried samples caused smearing during milling and thus the relationship between drying method and smearing should be studied further. All of the milled samples showed narrower particle size distribution compared to the Reference samples, which indicates a more homogenous flour and thus is considered a desirable trait in this study, when the specific food application is not known. Furthermore, all samples including the Reference samples showed negative skeweness in terms of their particle size distribution.

Avainsanat – Nyckelord – Keywords
House cricket, processing, drying, milling, grinding, edible insects

Ohjaaja tai ohjaajat – Handledare – Supervisor or supervisors
Laila Seppä

Säilytyspaikka – Förvaringställe – Where deposited
University of Helsinki, Digital Theses, E-thesis

Muita tietoja – Övriga uppgifter – Additional information
EKT Series 1905.

Tiedekunta – Fakultet – Faculty	Koulutusohjelma – Utbildningsprogram – Degree Programme
Maatalous-metsätieteellinen	Elintarviketieteiden maisteriohjelma

Tekijä – Författare – Author
Joonas Kauppinen

Työn nimi – Arbetets titel – Title
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Oppiaine/Opintosuunta – Läroämne/Studieinriktning – Subject/Study track
FOOD-600 Food Technology study module

Työn laji – Arbetets art – Level	Aika – Datum – Month and year	Sivumäärä – Sidoantal – Number of pages
Maisterintutkielma	Lokakuu 2019	42

Tiivistelmä – Referat – Abstract

Hyönteiset, kuten kotisirkat (*Acheta domesticus*) ovat ympäristöystävällinen vaihtoehto lihalle, kunhan niiden erityispiirteet, kuten allergisoivuus ja antinutrientit otetaan huomioon. Tämän maisterintutkielman tavoite oli tutkia kotisirkan prosessointimetodeja sekä –parametreja, joiden avulla voidaan valmistaa laadukasta ja turvallista sirkkajauhoa elintarvikesovellusten raaka-aineeksi. Tutkimusta voidaan käyttää referenssinä sirkkojen kuivaamista ja jauhamista varten tulevaisuudessa. Tavoite oli ensinnäkin selvittää optimaalinen kuivausaika uunilla sekä pakkaskuivurilla, jolla päästään alle 10 % vesipitoisuuteen sekä vedenaktiivisuuden vaihteluvälille 0,25-0,45. Näytteet, jotka täyttivät nämä laatuvaatimukset lyhimmällä mahdollisella kuivausajalla, jauhettiin kahdella eri seulakoolla, jonka jälkeen näytteistä mitattiin partikkelijakauma. Kuivattujen ja jauhettujen sirkkojen partikkelijakaumaa verrattiin uuni- ja pakkaskuivattujen, eri seulakoolla jauhettujen sekä kolmen eri yrityksen valmiiden sirkkajauhojen välillä. Kirjallisuudesta saatua 45-150 µm keskimääräistä partikkelikokoa käytettiin tavoitteena tässä tutkielmassa valmistettujen jauhojen partikkelikoolle. Optimaalinen kuivausaika 70 °C-asteessa uunikuivurilla oli 5 tuntia. Pakkaskuivatuille näytteille parametreilla Tp=25 °C, Tc=-87 °C, p=1 mbar optimaalinen kuivausaika oli 6 tuntia. Jauhetuista näytteistä 0,5 mm seulakoolla jauhetulla uunikuivatuilla jauholla oli alhaisin keskimääräinen ja mediaani partikkelikoko, kun puolestaan toisiksi pienin partikkelikoko oli 0,5 mm seulakoolla jauhetulla pakkaskuivatuilla jauholla. Siispä 5 tuntia uunikuivattu ja 0,5 mm seulakoolla jauhettu sirkkajauho oli lähimpänä tavoitetta, koska sillä oli pienin partikkelikoko. 1 mm seulakoolla jauhettujen jauhojen keskimääräinen ja mediaani partikkelikoko oli suurempi ja samaa kokoluokka kuin teollisuudesta saaduilla valmiilla sirkkajauhoilla. Lisäksi voidaan todeta, että 0,5 mm seulakoolla voidaan jauhaa hienojakoisempaa sirkkajauhoa, kuin mitä markkinoilla tällä hetkellä on saatavilla. Uunikuivatut näytteet kuitenkin aiheuttivat rasvan sulamisesta johtuvaa myllyn tahriintumista, jota pakkaskuivatuissa näytteissä ei havaittu. Siispä kuivausmetodin ja tahriintumisen suhdetta tulisi tutkia lisää. Verrattuna valmiisiin referenssijauhoihin, kaikilla näytteillä oli kapeampi partikkelijakauma, mikä viittaa homogeenisempaan jauhoon, jota voidaan yleisesti ottaen pitää positiivisena ominaisuutena.

Avainsanat – Nyckelord – Keywords
Kotisirkka, prosessointi, kuivaaminen, jauhaminen, hyönteiset

Ohjaaja tai ohjaajat – Handledare – Supervisor or supervisors
Laila Seppä

Säilytyspaikka – Förvaringställe – Where deposited
Helsingin yliopiston digitaaliset opinnäytteet, E-thesis

Muita tietoja – Övriga uppgifter – Additional information
EKT-sarja 1905.

Foreword

This Master's was done for the Department of Food and Nutrition at University of Helsinki as an independent project. No external funding or mandate was received for the thesis. Finnish food company Entocube provided the cricket material and all research was conducted at the University premises. This thesis was supervised by University lecturer Laila Seppä.

I would like to thank Laila Seppä for great guidance and supervision and Entocube for providing material for this thesis. I would also like to thank other University staff; University lecturer Kirsi Jouppila and Research technicians Mikko Kangas, Outi Brinck and Taru Rautavesi for introduction and guidance with the equipment used.

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1. Introduction

Environmental problems as well as food security for the ever-growing population are major global concerns of today's world. The Food and Agriculture Organization of the United Nations, FAO (2017) has given some clarity as to the extent of the challenges we are facing. Firstly, FAO has assessed that the livestock sector is one of the top three most significant contributors to our environmental issues (Steinfeld et al. 2006). Livestock emits more greenhouse gases than all transport systems worldwide and is directly or indirectly a major cause of e.g. ammonia emissions, deforestations, soil erosion, loss of plant biodiversity and water pollution (Steinfeld et al. 2006). These environmental concerns caused by food production tangle together with the issue of food security when the world population is estimated to rise to 9,8 billion by the year 2050 (FAO 2017). Therefore, one solution put forward by FAO as well as many researchers to mitigate both environmental devastation as well as famine is the use of edible insects, such as house cricket (*Acheta Domesticus*) as an alternative to animal protein, the way 2 billion people worldwide already do (Premalatha et al. 2011; Van Huis et al. 2013; Tabassum-Abbasi et al. 2016; van Huis and Tomberlin 2017).

What is the environmental impact of insect production then? There has been numerous studies on the subject and the results vary from extremely optimistic to more conservative and careful, depending on the way environmental impact is measured and based on the whole scope of the research. In terms of greenhouse gas (GHG) emissions, house cricket has been reported to emit only a fraction of the GHG of cattle (1,57 versus 2850 CO2 eq. g/kg mass gain, respectively) (Oonincx et al. 2010). With respect to Feed Conversion Ratio (FCR) i.e. the amount of feed needed to produce one kg of increased bodyweight, house cricket has been reported to need around the same amount of feed as poultry or even substantially less (1,7 kg vs 2,5 kg, respectively) (Van Huis et al. 2013; Oonincx et al. 2015). Van Huis et al. (2013) also noted that 80 % of the bodyweight of house cricket can be digested by humans whereas with cattle the same number is only 40 %, which increases the feed conversion efficiency of house cricket even further.

When environmental impact is measured by for instance Nitrogen Efficiency Conversion Index (N-ECI; %), Energy Efficiency Conversion Index (ECI) or Life Cycle Assessment (LCA), insects still seem to be superior to traditional livestock (Van der Hoek, Klaas W 1998; Collavo et al. 2005; van Broekhoven et al. 2015; Smetana et al. 2016; Salomone et al. 2017). Oonincx et al. (2015) found the N-ECI of house cricket to be 22-58 % whereas cattles nitrogen-efficiency was only 12 %, pigs 23 %

and broilers 33%. On the other hand when it comes to energy-efficiency, Collavo et al. (2015) stated that ECI of house cricket can be up to 92 %. Salomone et al. (2017) and Smetana et al. (2016) studied the environmental impact of insect production using Life Cycle Assessment - a more holistic and less specified method. Both studies concluded that insect production was more environmentally, e.g. 2-5 times more so than broiler according to Smetana et al. (2016). Van Broekhoven et al. (2015) and Smetana et al. (2016) also pointed out that insects could fit better to be fed with side streams from other branches of food industry, such as the mash from a brewery, compared to traditional livestock.

Producing ecological food is not beneficial unless it is nutritionally viable as well. This includes optimal amounts of total calories, protein, carbohydrates, fat, fibre and micronutrients (VRN 2014; WHO 2007). Furthermore, digestibility and essential amino acid content of the protein as well as the saturated/unsaturated fat ratio and essential fatty acid content are crucial to have balanced diet. Finke (2002) studied the energy content of house cricket and other edible insects and noted that the energy content was sufficient. Based on this and other studies done on various edible insects such as mealworms (*Tenebrio molitor*), insects do have sufficient amounts of dietary energy for human consumption (Finke 2002; Kouřimská and Adámková 2016; Nowak et al. 2016).

Total protein and essential amino acid content of edible insects has also been studied extensively (Jones et al. 1972; Ramos-Elorduy et al. 1997; Finke 2002; Collavo et al. 2005; Ghaly and Alkoaik 2009; Yi et al. 2013; Nowak et al. 2016; Payne et al. 2016; Zhao et al. 2016). For instance house cricket's protein content is 19,3 g/100 g of fresh weight according to Yi et al. (2013) and therefore equivalent to that of traditional livestock. Yi et al. (2013) also stated that there is sufficient amounts of all the essential amino acids in house cricket protein when guidelines of WHO (2007) are used as reference. Digestibility, a third critical indicator of proteins quality, has not been studied as comprehensively as the previous two. However, Ramos-Elorduy et al. (1997) analyzed the digestibility of 78 insect species and concluded that their digestibility was in the range of 76-98 %. Nevertheless, more studies should be done on digestibility and bioactivity of insect protein, especially when considering food supplement applications like insect based protein shakes for athletes (Burke 2001).

Collavo et al. (2005) showed that from house crickets total fat, up to 44,8 % is polyunsaturated fat and 22,2 % is monounsaturated fat, of which especially polyunsaturated fat content is fairly high. However it should be noted that even though polyunsaturated fat contains essential fatty acids, it is also more prone to oxidation, which should be considered when processing house crickets (Hsieh and Kinsella 1989). Hsieh and Kinsella (1989) studied especially fish, but polyunsaturated fat oxidation is assumed to apply to house crickets as well in this thesis

Insects contain high amounts of fiber called chitin (Collavo et al. 2005; Finke 2007; Van Huis et al. 2013). Chitin has been shown to possess characteristics typical for a dietary fibre, such as the ability to bind dietary cholesterol and triglycerides as well as improve the blood HDL-LDL-ratio (Koide 1998). Therefore house cricket is also an excellent source of fiber, because according to Finke (2002) it contains 6,8 g/100 g of chitin.

House crickets are also rich in vitamins and minerals (Finke 2002). Below in table 1 are micronutrient contents of house crickets regarding vitamins and minerals that Finnish people receive especially from animal sources, as well as total energy and protein content (VRN 2014). Examining the table it can be concluded not only that house crickets have sufficient amount of dietary energy and protein, but also higher contents of relevant micronutrients, excluding selenium, compared to broiler, pork and beef.

Table 1. Micronutrient-contents of house cricket, broiler, pork and beef in terms of vitamins and minerals most relevant to animal sources to Finnish people. Contents are expressed as kcal, g, µg or mg/100 g of fresh weight.

	House cricket	**Broiler**	**Pork**	**Beef**
Total energy	140,2 kcal[1]	120 kcal[2]	166 kcal[2]	201 kcal[2]
Protein	19,3 g[4]	22,5 g[2]	18,8 g[2]	20,3 g[2]
Selenium	19 µg[1]	23 µg[2]	27 µg[2]	22,9 µg[2]
Calcium	40,7 mg[1]	5 mg[2]	7 mg[2]	24 mg[2]
Iron	1,9 mg[1]	0,4 mg[2]	0,5 mg[2]	1,5 mg[2]
Zinc	6,7 mg[1]	0,7 mg[2]	1,7 mg[2]	3,6 mg[2]
Iodine	21 µg[1]	1 µg [3]	3 µg [3]	1,3 µg [3]
Riboflavin	3,4 mg[1]	0,2 mg[2]	0,2 mg[2]	0,1 mg[2]
B_{12}-vitamin	5,4 µg[1]	0,2 µg[2]	0,5 µg[2]	1,1 µg[2]

[1)](Finke 2002)
[2)](USDA 2017)
[3)](Fineli 2017)
[4)](Nowak ym. 2016)

After establishing the suitability of insects as food based on the environmental aspects as well as nutritional properties, the next question is often: Do insects pose a food safety risk? The food safety of insects has been studied quite widely and the short answer to the previous question is: The food safety risk-profile of insects is similar than that of traditional livestock with few exceptions (Sikorowski and Lawrence 1994; Witte 1998; Vijver et al. 2003; Collavo et al. 2005; Cappellozza et al. 2011; Klunder et al. 2012; Belluco et al. 2013; Rumpold and Schlüter 2013a; Rumpold and Schlüter 2013b; Rumpold et al. 2014; EFSA Scientific Committee 2015; Bußler 2016; Kouřimská

and Adámková 2016; van der Spiegel 2016; Houbraken et al. 2016; Poma et al. 2017). The first exception that separates crustaceans such as shrimps and house crickets from broiler, pork or beef is their pan-allergens or shared allergens (Schroeckenstein et al. 1990; Daul et al. 1994; Leung et al. 1996; Freye et al. 1996; Santos et al. 1999; Reese et al. 1999; Siracusa et al. 2003; Belluco et al. 2013; Bednárová et al. 2013; Rumpold and Schlüter 2013a; Rumpold and Schlüter 2013b; Verhoeckx et al. 2014). The allergenic potential is however more like a vital note to the food industry and the consumer than an argument against *entomophagy* or insect eating. The other exception arises from the fact that some insect species contain antinutrients (Thompson 1993; Koide 1998; Nishimune et al. 2000; Omotoso 2006; Ekop et al. 2010; Ifie and Emeruwa 2011; Rumpold and Schlüter 2013b). For instance, Nishimune et al. (2000) reported a case of thiamin deficiency in Nigeria due to consumption of insects containing thiaminase enzyme. The authors also pointed out however, that the adverse effects of thiaminase can be avoided with heat treatment. The problems caused by antinutrients are therefore highly relevant in developing countries where food security and nutrient deficiency are greater public health concerns (FAO 2017).

In order to create desirable food products from insect raw material, various processing steps need to be performed (Caparros Megido et al. 2016; Kouřimská and Adámková 2016; Alemu et al. 2017). Insect processing in general has been the subject of some studies (Omotoso 2006; Ghaly and Alkoaik 2009; Yi et al. 2013; Akpossan et al. 2015; Bußler 2016; Zhao et al. 2016; Jeon et al. 2016; Kim et al. 2016; Azzollini et al. 2016; Ssepuuya et al. 2017; Kröncke et al. 2018; Lenaerts et al. 2018). Kröncke et al. (2018) and Lenaerts et al. (2018) did study drying, water content and –activity of mealworms (*Tenebrio molitor*), but no such studies were found on house crickets. Since house crickets are more common insect material used in Finland, research about their behavior during drying is needed.

Water content and especially water activity needs to be decreased below 0,7 to inhibit microbe growth and below 0,45 to inhibit enzymatic activity (Labuza et al. 1972). However, water also shields lipids from oxidation, and thus lowering water activity below roughly 0,25 will increase lipid oxidation radically. Unsaturated, especially polyunsaturated fat is more prone to oxidation compared to saturated fat, which decreases the shelf life of products containing high amounts of unsaturated fat (Hsieh and Kinsella 1989). The decrease in shelf life is a result firstly from rancidity and other oxidation-caused deterioration of flavor, color and texture (Hsieh and Kinsella 1989). Secondly, when essential fatty acids oxidize, they lose their bioactivity (Hsieh and Kinsella 1989). Thirdly, harmful compounds can be produced in the oxidation process that may cause food safety hazard to the consumer (Hsieh and Kinsella 1989). House crickets contain high amounts of polyunsaturated fat,

and therefore water activity level should be between 0,25-0,45 to ensure optimal stability in terms of inhibiting fat oxidation as well as microbe growth and enzymatic activity (Labuza et al. 1972). Water content for ambient-stable house cricket flour was set at < 10 % in accordance with estimation for critical limit by van Huis and Tomberlin (2017).

Although optimal water content and –activity are determined more by universal food safety factors, particle size distribution is far from irrelevant. In fact, particle size is one of the most important factor affecting the quality aspects of bakery products made from flours (Russin et al. 2007; Chinma et al. 2007; Gómez et al. 2010; Zucco et al. 2011; Vishwanathan et al. 2011; de la Hera et al. 2013; Dhen et al. 2016). Furthermore, since this thesis has been justifying the use of house cricket as an environmentally friendlier alternative to meat, it could be justifiable to compare the specifications of house cricket flour to those of similar meat replacer: soy flour. For texturized vegetable protein products made by extrusion, Kearns et al. (1989) recommended that defatted soy flour with a mean particle size of 45-150 μm should be used to avoid problems. Kearns et al. (1989) point out that flour below these limits may be difficult to wet without lumping it, whereas too coarse flour would require complicated premoistening and would have chance of whole granules present in the finished product.

The goal of this Master's thesis is to study the processing methods and parameters of house cricket (*Acheta domesticus*) in creating flour for food applications that is safe and of optimal quality. The thesis hopes to provide a reference for parameters used in house cricket drying and milling, with the equipment available. Furthermore, the goal is to study the optimal time to get below critical value in water content of 10 % received from Huis and Tomberlin (2017) for insects in general and within critical values in water activity of 0,25-0,45 received from Labuza et al. (1972) for food products in general with an oven and a freeze-dryer. The samples within the critical safety values with minimal drying time are then milled with centrifugal mill with two different sieve-sizes and particle size distribution is measured and compared between oven-dried and freeze-dried and between the two sieve-sizes used. In addition, all measurements are done and results compared to three different reference house cricket flours received from the industry as well as to relevant literature. Although, the optimal particle size distribution of the flour depends on the food application, particle size range of 45-150 μm is used as a reference (Kearns et al. 1989).

2. Materials and methods

2.1. Preliminary tests

Preliminary tests were performed to optimize the drying times used at later stages of the thesis. Cricket samples were oven-dried and freeze-dried for 6 hours and water content and –activity was measured. Water content and –activity of Oven-dried sample were 2,28 ± 0,023 % and 0,33 ± 0,036, respectively. Water content and –activity of Freeze-dried samples were 1,44 ± 0,002 % and 0,24 ± 0,039, respectively. These results were used to receive a reference as to which drying times would be used in the thesis. If, for instance 6 hours of freeze-drying would result in very high water content and –activity, longer drying times would be used in the thesis, and vice versa. In this case the results were in line with the critical values determined for this thesis and thus the results could be used in the thesis.

2.2. Processing

Few process flowcharts have been suggested for insect processing (Arsiwalla and Aarts 2015; van Huis and Tomberlin 2017), which were used as inspiration. However, both of them involve fat separation due to problems with smearing. Thus, it is crucial to recognize that currently in Finland companies are allowed to sell only whole insects as food and not any fraction of them separately. This is due to the legal situation created by Finnish Food Authority's guideline 10588/2 (Finnish Food Authority 2018), which interpreted Regulation (EC) No 258/97 (EC 1997) in a way that allowed Finnish food companies to sell products with whole edible insects in them. Therefore, a process flowchart (figure 1), with necessary analysis for whole insects was created to give the reader a general view of the thesis.

All processing and analyzing steps were done with the equipment of the University of Helsinki. First, all insect material was decontaminated. Afterwards, processing steps were done to three separate batches and one measurement was done to each batch with each presented measurement result having n=3. The decision of having n=3 was made by examining similar studies (Lenaerts et al. 2018; Kröncke et al. 2018). The final result is presented as mean of those three batches ± standard deviation (n=3). If standard deviation was noted to be too high in some result, new measurements were done.

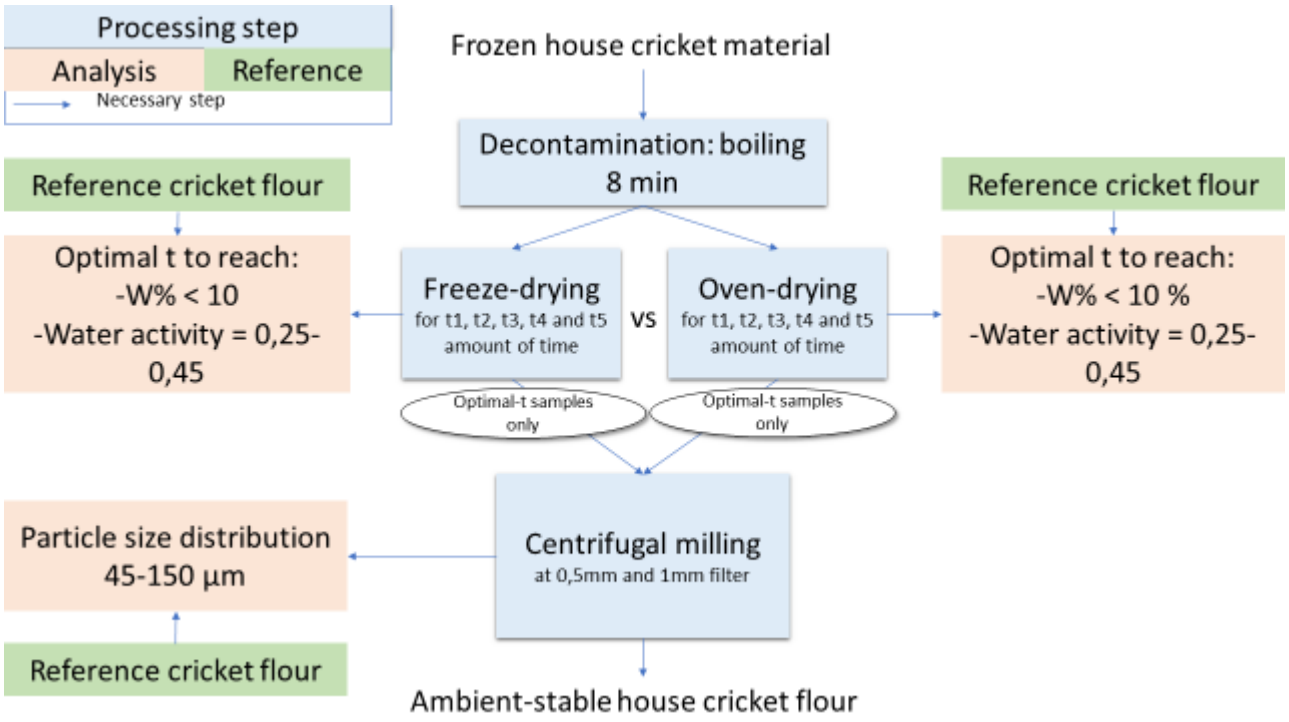

Figure 1. Schematic flowchart of the processes and analysis done in the thesis.

2.2.1. Insect samples and their preparation

House cricket material was provided to this thesis by Finnish insect company Entocube. House crickets were slaughtered by freezing, and consequently transported and stored frozen in -19 °C. All samples were decontaminated by boiling on a stove (Metos Ardox S2 400V3N, Metos, Finland). for 8 minutes and frozen back to -19 °C instantly to ensure microbiological stability as well as destruction of potential eggs, a worry expressed by University staff. Other decontamination methods were considered, but boiling and specifically 8 minutes were chosen based on relevant literature (Klunder et al. 2012; Rumpold et al. 2014; Bußler 2016; Caparros Megido et al. 2016). Since water content and water activity were measured in this study, the effect that boiling could have on these parameters was contemplated. However, the water content of boiled house cricket determined in this study (71,5 %) was very close to that of a fresh house cricket (69,2 %) reported by Finke (2002). The effect of boiling on the water activity could be interesting topic for further research. Three reference cricket flours were received for free from Entocube in addition to the frozen house cricket material.

2.2.2. Drying

Oven-drying was done in an oven (TERMAKS B8260 Incubator, Termaks, Norway) at 70 °C. This is similar to temperature range of 50-70 °C used in the literature for air tunnel drying of mealworms (Azzollini et al. 2016). Because the water content of 3-hour oven-dried sample was below 10 %, an additional 2-hour oven-dried sample was prepared in order to observe the point where the water content falls below critical value

Before freeze-drying, the samples were moved to a freezer to -75 °C for 24 hours. Freeze-drying was done with a freeze-dryer (Dura Dry Freeze Dryer, FTS Systems, USA) in stable conditions with plate temperature of 25 °C, coil temperature of -87 °C and chamber pressure of 1 mbar, which are standard parameters used at the University.

2.2.3. Milling

Centrifugal milling was done (Ultra Centrifugal Mill ZM 200, Retsch, Germany) with two sieve sizes: 0,5 mm and 1 mm. Speed at 50 Hz of 10 000 rpm was used, based on the recommendation by University staff. Oat flour, which has similar fat profile has been milled with the same equipment before and it caused smearing in higher milling speeds due to heat caused by friction, according to University staff. A reference of milling speed used for house cricket material was not found from literature, but because for instance Bußler (2016) and van Huis and Tomberlin (2017) discussed about fat smearing during milling of insects, a speed of 10 000 rpm was used based on University staff recommendation in this thesis as well.

During milling notable smearing happened only for oven-dried samples and not to freeze-dried samples. Examples of this can be observed from picture 1a where dark paste can be found in the sieve after milling the oven-dried sample.

Similar paste did not form when milling the freeze-dried sample (picture 1b). This difference could be explained with the lower water content and -activity of the freeze-dried sample (1,44 vs 2,06 and 0,24 vs 0,38, respectively), observable from the section 3.1. of this thesis. Since fat content was not measured in this thesis, the samples could also have different fat profiles, although they were taken from the same batch of raw material.

Picture 1a. Paste formed from smearing of oven-dried samples.

Picture 1b. Paste not formed when milling freeze-dried samples.

2.3. Analysis

2.3.1. Water content

Water content was determined by drying and weighing (Presica 1000C - 3000D, Presica, Switzerland) the samples and calculating the water content from the weight loss. All weight loss was presumed to be water, although small amounts of volatile compounds most likely also evaporated from the material. Final water content was determined by drying the insect samples at 70 °C or until stable weight was reached. Freeze-drying was done in plastic containers and thus water removal through drying for the final water content determination was done in lower temperature of 45 °C in air-dryer (PERUS ORAKAS, Orakas Tuotteet, Finland) in an airflow of 1,58m/s. The airflow was measured with Thermo-anemometer (Alnor GGA-6, Alnor, Poland). Standard University oven normally used for water content measurement was not available due to renovation, which is why the equipment mentioned above were used.

2.3.2. Water activity

After drying the samples were immediately sealed in to plastic measurement containers to avoid moisture resorption. Three house crickets were put in to each container to improve comparability of the results. Water activity was measured (Novasina LabMaster, Novasina, Switzerland) until stable value was reached.

2.3.3. Particle Size distribution

Particle size distribution for the milled house cricket flour was measured using static light scattering instrument (Mastersizer 3000, Malvern Instruments, UK) with dry sample dispersion.

Same parameters were used for all samples unless stated otherwise. The parameters were set based on consultation of University staff and by studying the user manual (Malvern Instruments 2013). Particle Refractive Index of 1,53 was used, which is the Particle Refractive Index of faba bean flour. Particle Absorption Index and Dispersant Refractive Index was 1. Scattering model used was Mie and Analysis model was General Purpose. Non-spherical sample type was set from options. Venturi type was standard venturi dispenser, tray type was general purpose tray with hopper. Measurement obscuration filtering was enabled with 10-second time out, in order to include only data within obscuration limits, which improves repeatability. Measurement obscuration limits were 0,1 % for lower limit and 6 % for higher limit to ensure stable sample feed and thus accurate measurements. Measurement were set to auto start when obscuration is in range with stabilization time of 0,1 seconds. 10-second background measurement and 30-second sample measurement duration was set.

Reference 1 was done with feed rate of 60 % and hopper gap of 3,5 mm. However, problems occurred with Reference 2 and 3 in terms of sample feed. Therefore feed rate was increased to 80 % and hopper gap to 4 mm and these parameters were used to all remaining samples. Besides this, data quality was good according to Mastersizer software and no further problems occurred during measurements.

Weighted residual, laser obscuration and date of measurement are indicated in a table below (table 2). According to Malvern Instruments (2013) Weighted Residual indicates the degree to which calculated data was fitted to the measurement data with a residual of under 1 % representing a good fit. On the other hand, Weighted Residual of over 1 % may be a sign of the use of incorrect absorption values and refractive index for the sample and dispersant. Laser Obscuration measures the amount of laser light in per cent lost as a result of the introduction of the sample into the analyser beam. Formula

for calculating Laser Obscuration can be found from appendix 2 as formula 1. All formulas used by the light scattering instrument can be found in appendix 2.

Table 2. Sample-specific measurement parameters of particle size distribution analysis (n=3) ± standard deviation.

Sample	Weighted Residual (%)	Laser Obscuration (%)	Date
Reference 1	0,82 ± < 0,001	0,31 ± 0,003	23.4.2019
Reference 2	1,03 ± < 0,001	0,90 ± 0,004	13.6.2019
Reference 3	0,51 ± < 0,001	2,08 ± 0,001	13.6.2019
Oven-dried 0,5	0,55 ± < 0,001	2,49 ± 0,001	24.6.2019
Oven-dried 1	0,70 ± < 0,001	2,09 ± < 0,001	24.6.2019
Freeze-dried 0,5	0,63 ± < 0,001	2,47 ± 0,003	24.6.2019
Freeze-dried 1	0,76 ± < 0,001	0,174 ± 0,002	24.6.2019

2.3.4. Statistical analysis

Statistical analysis was done with IBM SPSS Statistics 25 – statistical software. The measurement data was compared between drying times and methods with one-way analysis of variance followed by the post-hoc Duncan test was performed in case variances were equal and Kruskal-Wallis H test including pairwise post-hoc test with adjusted significance by Bonferroni correction. Homogeneity of variance was tested with Levene test. Furthermore, a Pearson bivariate correlation was calculated for water content and –activity. A 95 % confidence level was presumed with a statistical significance level of $p < 0,05$. All results presented are presented as a mean of three separate measurements from three separate batches (n=3) ± standard deviation.

3. Results

In picture 3 there are examples of the dried samples. No clear pattern can be observed in color between the oven-dried vs freeze-dried samples or between samples dried for different amounts of time. However, sample color could be assumed to darken when drying temperature or time increases and this could be a topic for further research with house crickets.

Picture 3. Oven- and Freeze-dried house cricket samples.

3.1. Water content and -activity

Below in table 3 are water contents and –activities of all the samples in this study. It is important to note that the reference-samples were in the form of flour whereas the samples studied were in the form of whole house crickets at this point of the study. Therefore the water content and –activity of the samples should be compared to critical values (w-% < 10 % and A_w = 0,25-0,45) determined in the Introduction-section and not to the reference samples. Whole house crickets would probably trap more moisture within their structure. Based on the criteria of water content < 10 % and water activity of 0,25-0,45, Oven-dried 5 h samples and Freeze-dried 6 h samples were chosen for further processing, because these samples should have optimal quality in terms of microbial- and enzymatic activity as well as fat oxidation.

Drying time and –method affected the water content and –activity of the samples statistically significantly ($p < 0,05$). Water content and -activity results, which varied statistically significantly ($p < 0,05$) can be observed from the superscripts from table 3.

Table 3. Measured water contents and –activities (n=3) ± standard deviation.

Sample	Water content (%)	Water Activity	Date
Reference 1	$5,56^{a,b} \pm 0,002$	$0,34^{c,d} \pm 0,05$	12.6.2019
Reference 2	$2,82^{a} \pm 0,003$	$0,27^{b,c,d} \pm 0,02$	12.6.2019
Reference 3	$3,56^{a,b} \pm 0,003$	$0,34^{c,d} \pm 0,009$	12.6.2019
Undried	$71,52^{d} \pm 0,009$	$0,94^{g} \pm 0,02$	7.6.2019
Oven-dried, 2h	$18,82^{c} \pm 0,02$	$0,72^{e,f} \pm 0,07$	10.6.2019
Oven-dried, 3h	$3,39^{a,b} \pm 0,01$	$0,66^{e} \pm 0,1$	11.6.2019
Oven-dried, 4h	$1,15^{a} \pm 0,004$	$0,42^{d} \pm 0,09$	10.6.2019
Oven-dried, 5h	$2,06^{a} \pm 0,009$	$0,38^{c,d} \pm 0,1$	12.6.2019
Oven-dried, 6h	$2,28^{a} \pm 0,02$	$0,33^{c,d} \pm 0,04$	7.6.2019
Oven-dried, 18h	$0,13^{a} \pm 0,001$	$0,13^{a,b} \pm 0,008$	11.6.2019
Freeze-dried, 3h	$21,68^{c} \pm 0,04$	$0,92^{g} \pm 0,02$	12.6.2019
Freeze-dried, 4h	$17,64^{c} \pm 0,05$	$0,89^{f,g} \pm 0,04$	12.6.2019
Freeze-dried, 5h	$8,89^{b} \pm 0,05$	$0,66^{e} \pm 0,06$	12.6.2019
Freeze-dried, 6h	$1,44^{a} \pm 0,002$	$0,24^{a,b,c} \pm 0,04$	11.6.2019
Freeze-dried, 18h	$0,27^{a} \pm 0,001$	$0,09^{a} \pm 0,05$	10.6.2019

^{a,b,c,d,e,f,g}Values in the same column with a different superscript differ significantly ($p < 0,05$).

In a meeting about the results, as the standard deviation of the water content of Oven-dried 6h sample was abnormally high it was decided that a new water content and –activity measurement was done for Oven-dried 6h sample. This time three separate batches were dried and three analyses done for each batches with the sampling of each result being n=9 instead of n=3, like the rest of the samples. The water content and –activity of the new Oven-dried 6h sample was 2,35 ± 0,004 % and 0,31 ± 0,06, respectively, which was more in line with the rest of the results in terms of standard deviation of the water content. The new Oven-dried 6h sample varied significantly ($p < 0,05$) from the Undried, Oven-dried 2h as well as Freeze-dried 3h, 4h and 5h samples in terms of water content. In terms of water activity it varied significantly ($p < 0,05$) from the Undried, Oven-dried 2h, 3h and 18h as well as Freeze-dried 3h, 4h, 5h and 18h samples.

Higher, statistically significant ($p < 0,001$) correlation was observed between water content and water activity of Oven-dried samples with correlation coefficient of 0,774 when compared to that of Freeze-

dried samples ($p < 0{,}005$) with correlation coefficient of 0,675. The correlation coefficient ($p < 0{,}001$) for all samples combined was 0,699. Whether or not this higher correlation is relevant in predicting the food safety of the material, could not be found from the literature.

The full 18-hour figure of water content is depicted as a function of time in figure 2. In figure 3, water content is depicted until 6 hours for the readers convenience.

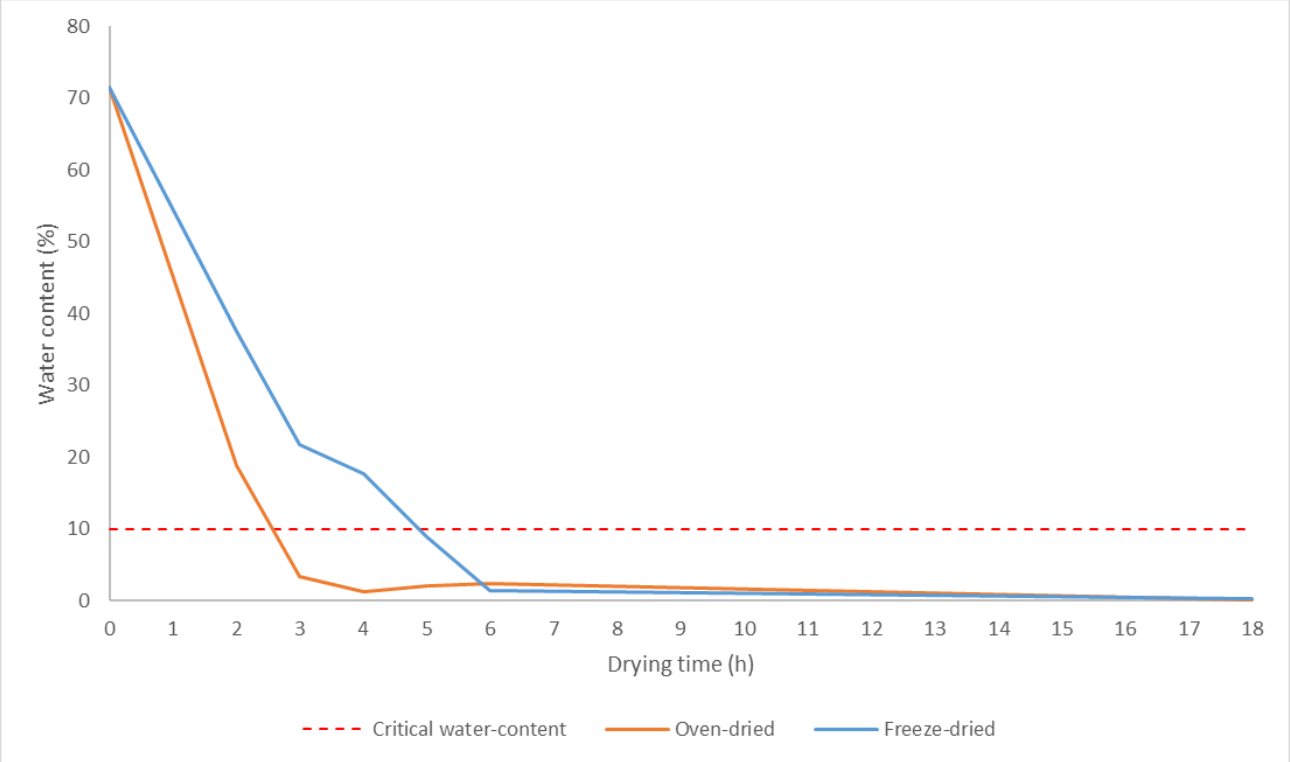

Figure 2. Water content of dried house crickets for full 18 h period.

Theoretical critical drying time, i.e. the drying time in between measurements when water content falls below 10 % can be calculated if the drying is assumed to happen linearly in between the measurements, as depicted in the figure. The drying time can be calculated using the formula below (2), where *tf* is the amount of full hours measured before water content decreases below critical level, *wc before* is the last water content measured before it decreases below critical level, *critical wc* is the critical water content and *wc after* is the first water content measured after it decreases below critical level.

$$Theoretical\ critical\ drying\ time\ = tf\ + \frac{wc\ before\ -\ critical\ wc}{wc\ before\ -wc\ after} \quad (2)$$

With this formula the theoretical critical drying time in terms of water content is 2,57 hours for oven-drying and 4,87 hours for freeze-drying.

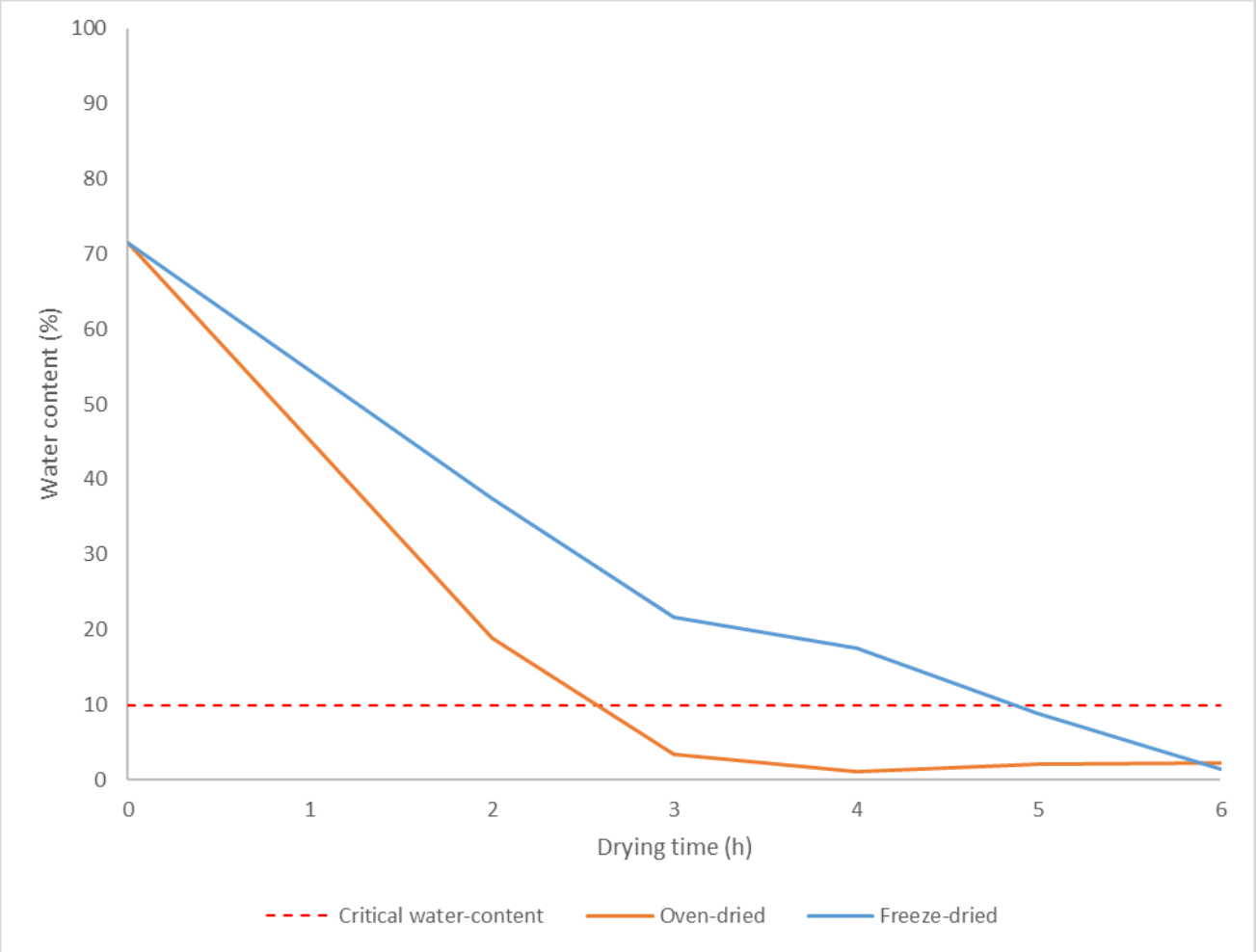

Figure 3. Water content of dried house cricket samples as a function of drying time.

Below is a figure of drying time in terms of water activity (figure 4). Theoretical critical drying time could be calculated for the samples in terms of water activity as well with formula (3) which would be used separately for minimum and maximum drying times. In formula (3) *tf* is the amount of full hours measured before water activity decreases below critical level, *aw before* is the last water activity measured before it decreases below critical level, *critical aw* is the critical water activity and *aw after* is the first water activity measured after it decreases below critical level. Optimal drying time for oven-drying would be 3,89-10,39 hours and for freeze-drying 5,50-5,97 hours.

$$Theoretical\ critical\ drying\ time\ = tf\ + \frac{aw\ before\ -\ critical\ aw}{aw\ before\ -aw\ after} \quad (3)$$

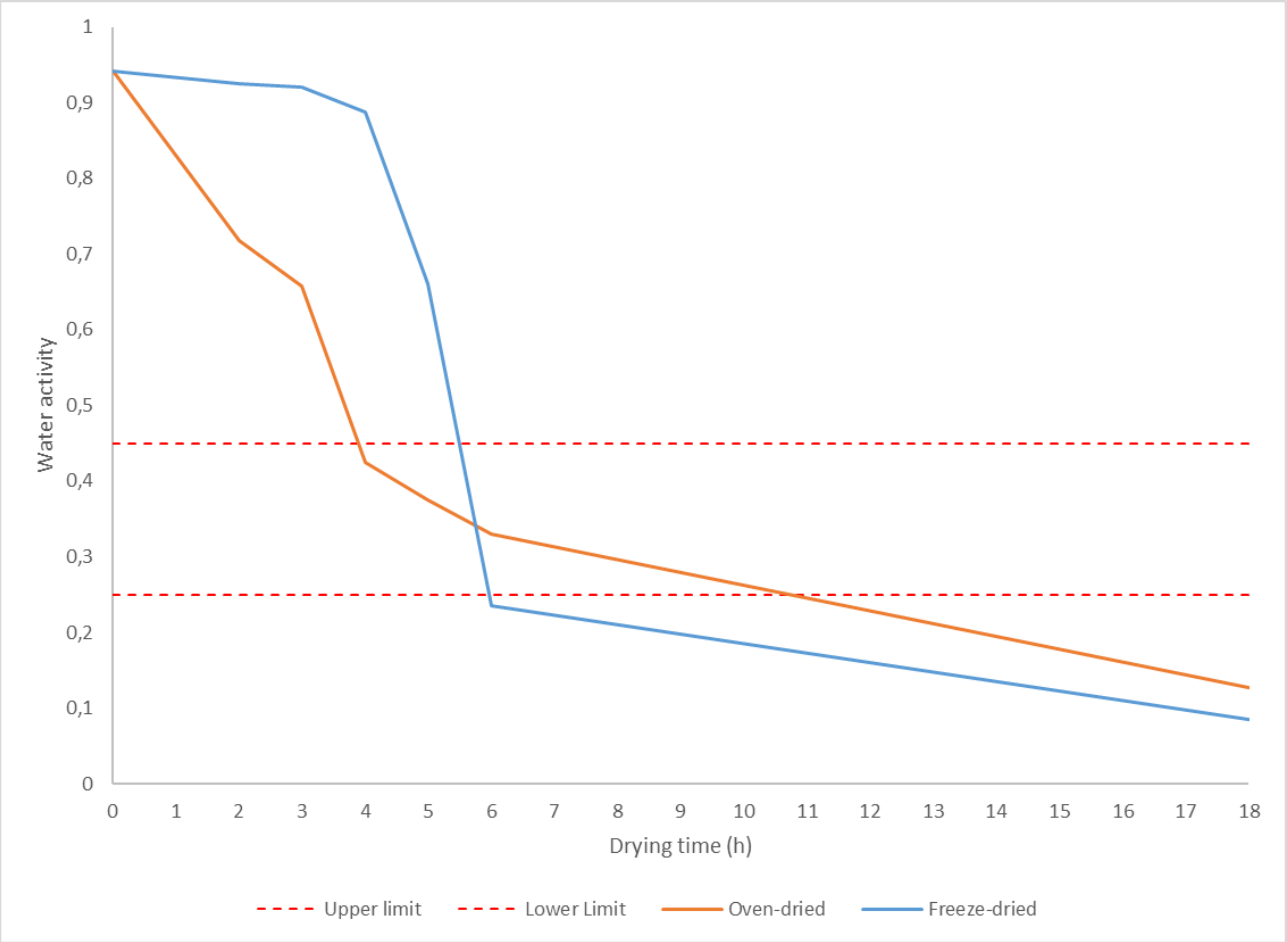

Figure 4. Water activity as of dried house cricket samples as a function of drying time.

3.2. Particle size distribution

Below is a picture of milled samples (Picture 4). Freeze-dried sample ground with 0,5 mm sieve size looks much more light in color than the other samples for some unknown reason. The samples looked similar before milling as can be observed from picture 3 and the reason for the lighter color is not known. Instrumental color analysis and various sensorial analyses could be good topics for further research. As said in section 2.1.3. Reference 2 and 3 –samples moved poorly during particle size distribution measurement. Furthermore, they also seemed prone to caking when they were handled during the analyses.

Picture 4. Milled house cricket flours.

Below in table 4 is the first part of the results of particle size distribution measurement. The results of particle size distribution measurements are divided in to two separate tables due to high amount of information and thus lack of space in the table. According to Malvern Instruments (2013) Concentration is the volume concentration of the sample, whereas span indicates the width of the distribution: the narrower the distribution becomes, the more the span decreases. Uniformity is a measure of the absolute deviation from the median. Specific surface area is the total area of the particles divided by the total weight (Malvern Instruments 2013). All of the results in table 4 showed no statistically significant variance ($p > 0,05$). The formulas for calculating Concentration, Span, Uniformity and Specific surface area, can be found from formulas 4, 5, 6, and 7, respectively from appendix 2.

Table 4. Part one of results of particle size distribution measurements (n=3) ± standard deviation.

Sample	Concentration (%)	Span	Uniformity	Specif. surf. area (m^2/kg)
Reference 1	0,02 ± < 0,001	1,99 ± 0,03	0,62 ± 0,009	55,16 ± 0,5
Reference 2	0,02 ± < 0,001	2,24 ± 0,05	0,69 ± 0,001	28,54 ± 1,1
Reference 3	0,03 ± < 0,001	2,09 ± 0,02	0,64 ± 0,006	54,03 ± 0,3
Oven-dried 0,5 mm	0,03 ± < 0,001	1,83 ± 0,04	0,57 ± 0,01	55,02 ± 0,4
Oven-dried 1 mm	0,05 ± < 0,001	1,74 ± 0,006	0,54 ± < 0,001	32,96 ± 1,04
Freeze-dried 0,5 mm	0,04 ± < 0,001	1,94 ± 0,02	0,60 ± 0,006	53,37 ± 1,2
Freeze-dried 1 mm	0,04 ± < 0,001	1,88 ± 0,02	0,58 ± 0,007	33,90 ± 1

Below in table 5 is the second part of the particle size distribution measurement results. D[3,2] is the surface weighted mean particle diameter and D[4,3] is the volume weighted mean particle diameter. The generalized formula for calculating surface- and volume weighted mean particle diameter can be found from formula 8 in appendix 2. The fundamental size distribution derived by Laser diffraction methods, used in this thesis is volume-based and thus Volume weighted mean particle diameter D[4,3] is used in this thesis to represent the particle size of the flour (Malvern Instruments 2013; Horiba Instruments 2017). Dx(10), Dx(50) and Dx(90) are standard 10 %, 50 % and 90 % percentile readings, respectively, from the analysis, i.e. particle sizes equivalent to the cumulative distribution (Malvern Instruments 2013). For instance, Dx(10) represents the value below which 10 % of the cumulative distribution lies. Dx(50) is therefore also the median of the sample.

Table 5. Part two of results of particle size distribution measurements (n=3) ± standard deviation.

Sample	[D3,2] (µm)	D[4,3] (µm)	Dx(10) (µm)	Dx(50) (µm)	Dx(90) (µm)
Reference 1	109^{a} ± 1	262^{c} ± 3,5	$49,2^{a,b}$ ± 0,5	235^{c} ± 1	517^{c} ± 9,7
Reference 2	210^{c} ± 8,5	460^{f} ± 23	104^{e} ± 5,7	373^{f} ± 15,01	940^{f} ± 52,5
Reference 3	111^{a} ± 1	241^{b} ± 3,5	$45,6^{a}$ ± 0,2	208^{b} ± 2,08	$481^{b,c}$ ± 8,4
Oven-dried 0,5 mm	109^{a} ± 1	210^{a} ± 1,2	$53,1^{b}$ ± 1,08	189^{a} ± 0,6	398^{a} ± 4,9
Oven-dried 1 mm	182^{b} ± 6,2	319^{d} ± 9,5	$99,2^{d}$ ± 3,2	282^{d} ± 8,2	590^{d} ± 18,3
Freeze-dried 0,5 mm	112^{a} ± 2,3	232^{b} ± 4	$53,4^{b}$ ± 1,2	205^{b} ± 4	451^{b} ± 6,4
Freeze-dried 1 mm	177^{b} ± 5,5	348^{e} ± 12,7	$90,3^{c}$ ± 2,2	305^{e} ± 10,7	664^{e} ± 25,9

^{a,b,c,d,e,f}Values in the same column with a different superscript differ significantly ($p < 0,05$).

In terms of volume weighted mean particle diameter D[4,3], oven-dried sample milled with 0,5 mm sieve size had the lowest particle size, 210 ± 1,154 µm and varied significantly ($p < 0,05$) from all

other samples. The second lowest was freeze-dried sample milled with 0,5 mm sieve size with particle size of 232 ± 4,041 μm, which varied significantly ($p > 0,05$) from all other samples, except for Reference 3.

Both samples milled with 1 mm sieve size had larger particle size than Reference 1 and 3 but smaller than Reference 2, Oven-dried sample having again smaller particle size compared to Freeze-dried sample (319 ± 9,45 μm vs 348 ± 12,74 μm, respectively). Both samples milled with 1 mm sieve size varied significantly ($p < 0,05$) from each other and all other samples. As to be expected, both samples milled with smaller sieve size had lower mean particle size, because the sieve should restrict the entry of larger particles in to the matrix of the end product inside the mill.

Median or Dx(50) in table 5 is also one of the most meaningful statistics for particle size distributions (Horiba Instruments 2017). All of the samples had around 20-90 μm smaller median in compared to their mean particle size, which indicates that the particle size distributions are not normally distributed. In terms of median particle size, the Oven-dried 0,5 mm sample was again the finest (189 ± 0,577 μm), followed by Freeze-dried 0,5 mm sample (205 ± 4 μm), Reference 3 and 1 (208 ± 2,08 and 235 ± 1 μm, respectively), Oven- and Freeze-dried 1 mm samples (282 ± 8,19 and 305 ± 10,69 μm, respectively) and finally Reference 2 (373 ± 15,011 μm). All of the samples varied significantly from each other ($p < 0,05$), except for Freeze-dried 0,5 mm sample and Reference 3 ($p > 0,05$).

Particle size distribution measurement results including all samples can be found from Appendix 2. However, with too many results depicted in one figure, the results become more difficult to interpret and thus, for readers convenience, the results are presented in three separate figures. The mode, which according to Horiba Instruments (2017) is the peak of the frequency distribution, was observed from the measurement data and is presented below. The width of the distribution is represented by Span in table 4.

Below in figure 5 are particle size distributions of oven-dried samples compared to freeze-dried samples. As with mean and median, also the mode of both Oven-dried –samples is lower, with 8,93 % of the particles of Oven-dried 0,5 mm –sample being 256-290 μm and 9,41 % of the particles of Oven-dried 1 mm –sample being 330-375 μm in size. Conversely, 8,38 % of the particles of Freeze-dried 0,5 mm –sample were 290-330 μm in size and 8,69 % of the particles of Freeze-dried 1 mm –sample were 375-423 μm in size.

In terms of width of the distribution, Oven-dried 0,5 mm had narrower and taller distribution than Freeze-dried 0,5 mm sample with span of 1,83 and 1,94 respectively. Oven-dried 1 mm had also

narrower and taller distribution than Freeze-dried 1 mm –sample, with span of 1,74 and 1,88 respectively.

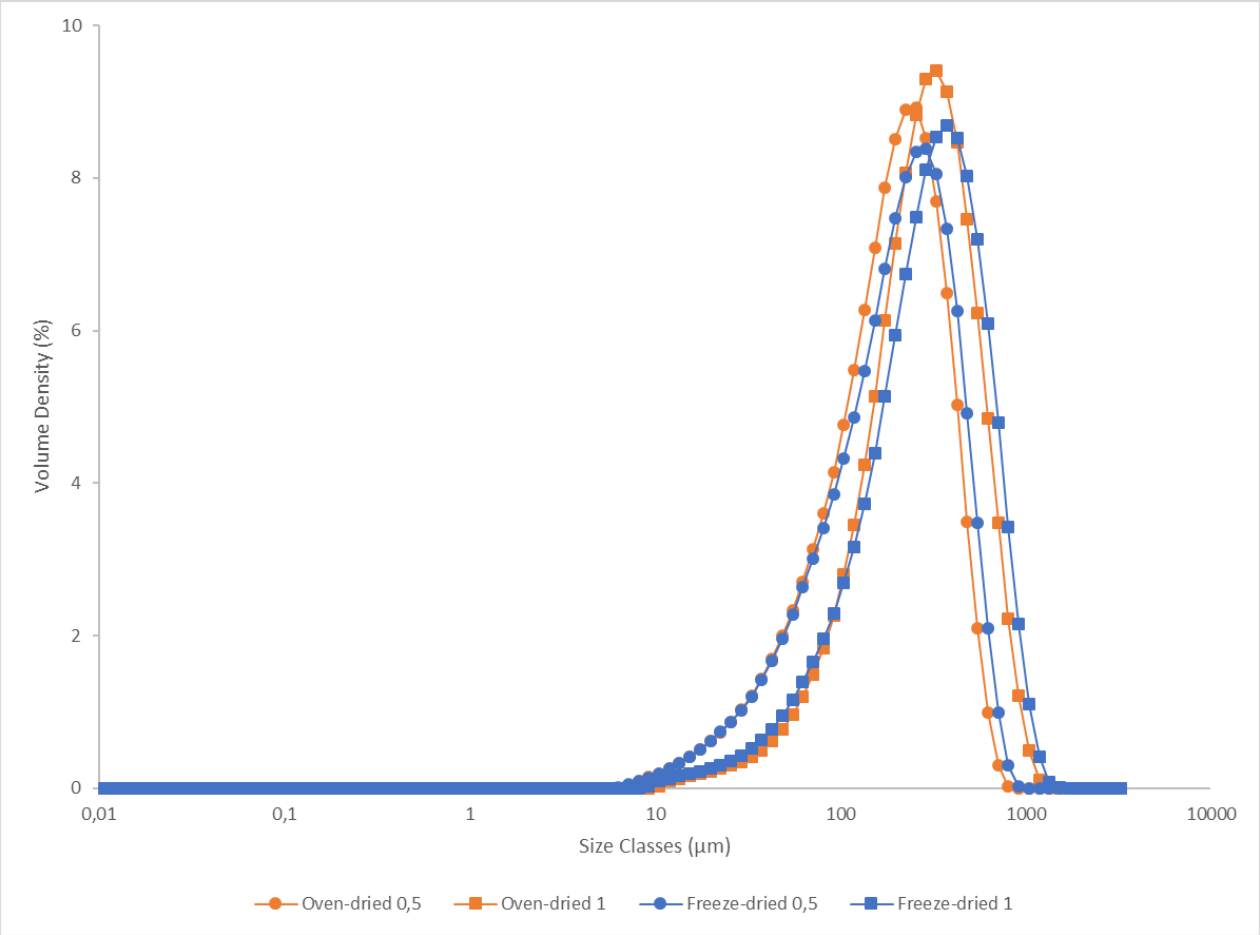

Figure 5. Particle size distributions of oven-dried and freeze-dried samples.

Below in figure 6 are oven-dried samples compared to reference samples. 8,39 % of the particles of Reference 1 were 330-375 µm; 7,56 % of the particles of Reference 2 were 426-484 µm and 8,19 % of the particles of Reference 3 were 256-290 µm in size.

Furthermore, the most abundant particles of Oven-dried 0,5 mm –samples were the same size as those of the particles of Reference 3 (256-290 µm both), but smaller than those of the particles of Reference 1 (330-375 µm) and 2 (426-484 µm). The most abundant particles of Oven-dried 1 mm sample were the same size as those of the particles of Reference 1 (330-375 µm both); smaller than those of Reference 2 (426-484 µm) and larger than those of Reference 3 (256-290 µm). The distribution of both of the oven-dried samples was narrower and taller than those of the reference samples, with Span being 1,99 for Reference 1; 2,24 for Reference 2 and 2,09 for Reference 3.

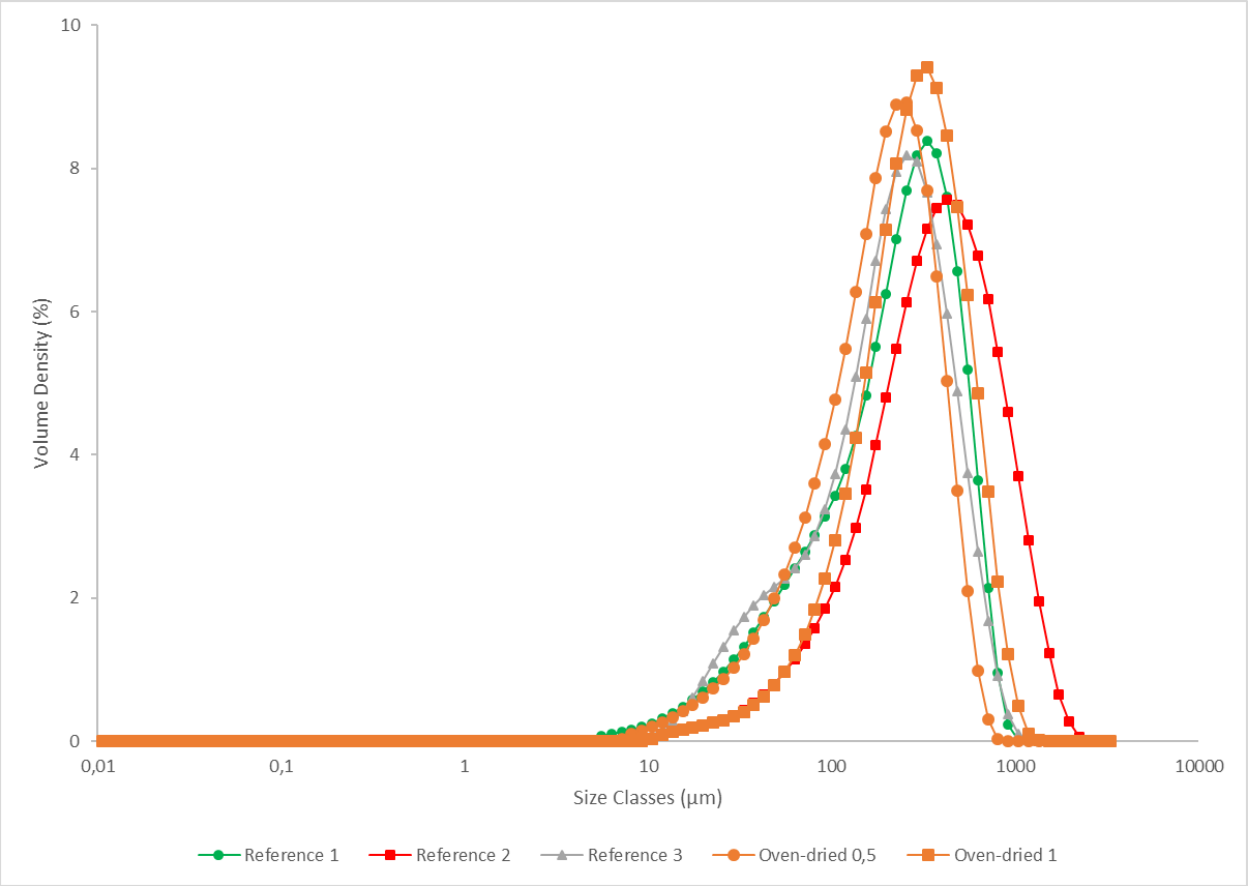

Figure 6. Particle size distributions of oven-dried samples in comparison to reference samples.

Below in figure 7 are freeze-dried samples compared to reference samples. When comparing figure 5 and 6 as well as the numerical representations of the modes and Span, it can be observed that the particle size distribution of the freeze-dried samples were more similar to those of the reference samples than what the particle size distributions of the oven-dried samples were.

The distribution of Freeze-dried 0,5 mm –sample was taller with the mode at 8,38 % than that of Reference 2 (7,56 %) and 3 (8,19 %), but shorter than that of Reference 1 (8,39 %), with the most abundant particles being larger (290-330 µm) than that of Reference 3 (256-290 µm), but lower than that of Reference 1 (330-375 µm) and 2 (426-484 µm).

The distribution of Freeze-dried 1 mm –sample was taller with mode at 8,69 % than all of the reference samples, with the most abundant particles being larger (375-423 µm) than that of Reference 1 (330-375 µm) and 3 (256-290 µm), but lower than that of Reference 2 (426-484 µm).

As with the Oven-dried samples, the distribution of the Freeze-dried samples was also narrower than all of the reference samples, as can be observed from figure 6 and Span from table 4. This indicates

that all of the dried samples used in this thesis were more homogenous than the reference samples in terms of particle size distribution.

All of the samples showed a pattern of the mode > mean of the particle size, which indicates negative skeweness. This can also be observed from the thicker left tail compared to the right tail in all distributions in figures 4, 5 and 6.

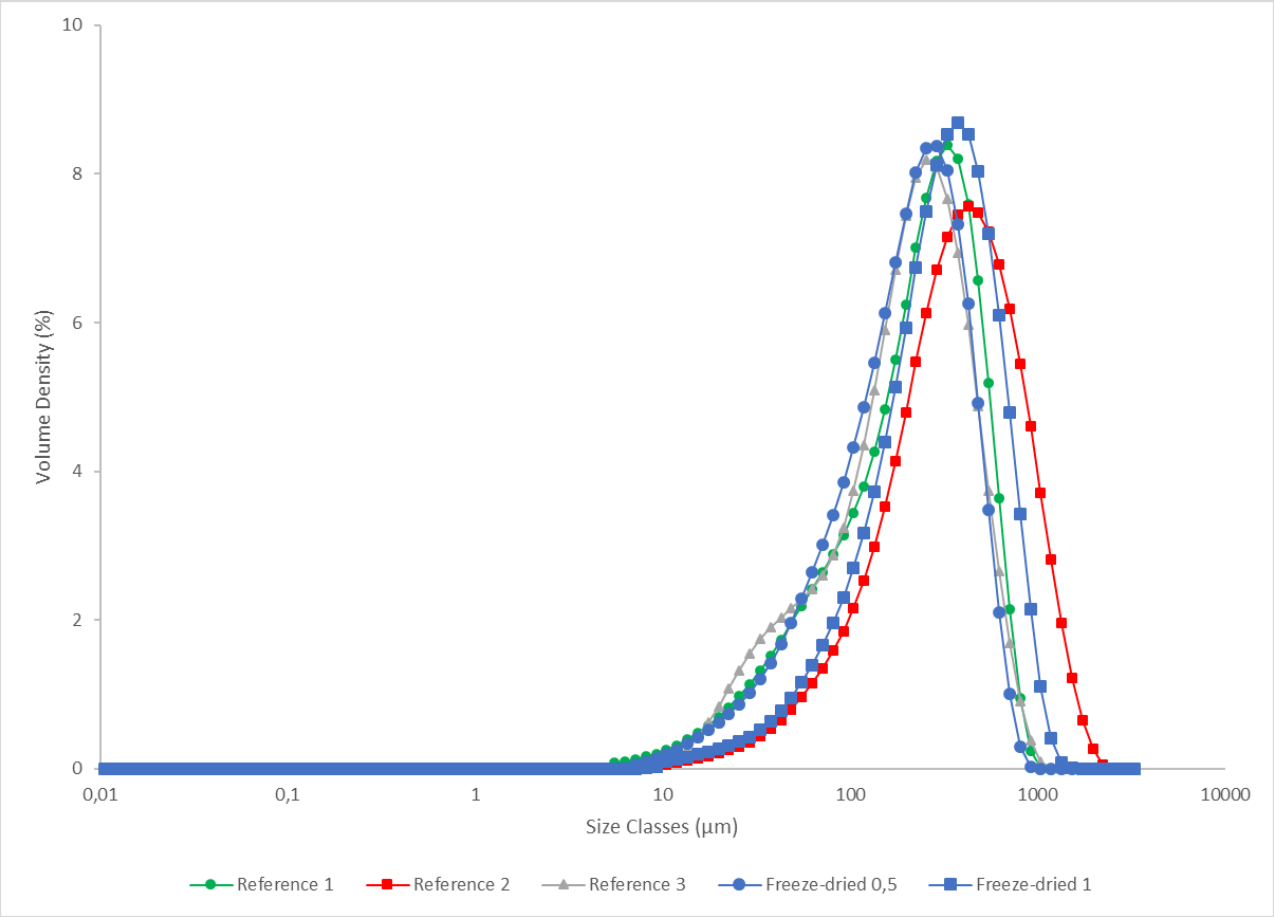

Figure 7. Particle size distributions of freeze-dried samples in comparison to reference samples.

4. Discussion

4.1. Water content and -activity

As mentioned in Introduction, Lenaerts et al. (2018) and Kröncke et al. (2018) studied the effect of various drying processes on some properties of mealworms (*Tenebrio molitor*). Lenaerts et al. (2018)

compared microwave drying to freeze drying with and without blanching and vacuum, water content and activity was measured, and other analysis such as vitamin B12 content, oxidation status was determined. Lenaerts et al. (2019) had a water activity target level below 0,6 without a lower boundary in contrast to water activity limits of this thesis (0,25-0,45). Lenaerts et al. (2018) reported a water content and –activity levels of blanched mealworms to be 66,36 ± 0,47 % and 0,98 ± 0,00, respectively, which is fairly similar than the water content and –activity of blanched, undried samples obtained in this study (71,52 ± 0,009 % and 0,94 ± 0,02, respectively). Lenaerts et al. (2018) also showed that blanching did not raise water content and activity remarkably (from 62,45 ± 0,07 % to 66,36 ± 0,47 % and from 0,99 ± 0,00 to 0,98 ± 0,00, respectively), which was also the observation of this study when comparing the water content of blanched house crickets to the fresh ones from Finke (2002).

Kröncke et al. (2018) also studied alternative drying methods to freeze-drying and did analysis on water content and activity of the mealworms, as well as the effect of different drying times. Most interesting in relation to this study were their preliminary studies with rack-oven drying and freeze-drying. In terms of oven-drying (RI 1,0608-TL, MIWE, Germany), they reached a water content of 21,81 % and water activity level of 0,78 in 2 hours and water content level of 8,39 % and water activity level of 0,61 in 4 hours at 60 °C. The oven-dried samples dried in this study showed similar water content and –activity of 18,82 ± 0,02 % and 0,72 ± 0,07 at 2 hours but lower water content and –activity levels of 1,15 ± 0,004 % and 0,42 ± 0,09 at 4 hours, respectively. Here it is crucial to remember that the material and the drying temperature was different, which affects the results.

In terms of freeze-drying (Christ Beta 1-8, Martin Christ, Germany), Kröncke et al. (2018) first froze the samples to -21 °C and then freeze-dried them with condenser temperature of -50 °C in a vacuum at unknown pressure, not specified in the article. They reached a water content level of 15,21 % and water activity level of 0,71 in 12 hours and water content level of 3,69 % and water activity level of 0,25 in 24 hours. In this study, a water content level of 1,44 ± 0,002 % and water activity level of 0,24 ± 0,04 was reached in only 6 hours. Again, the drying temperature and material was different and the pressure could not be compared, since Kröncke et al. (2018) did not report it. Also, Kröncke used 350 g batches, whereas in this study the batch size was not standardized and was also much smaller (few dozen grams) due to small drying containers available. In the industrial scale the amount of material would be much larger, but also the equipment would be different and thus it is hard to compare the results.

4.2. Particle size distribution

As said in the Introduction, defatted soy flour with particle size of 45-150 μm is recommended to be used for texturized vegetable protein products produced by extrusion (Kearns et al. 1989). Kearns et al. (1989) pointed out that wetting a powder with particle size < 38 μm without lumping it would be very difficult. Furthermore, coarse flour with particle size > 180 μm would require complicated premoistening and whole granules would often be seen in the finished product. As pointed out in Section 2 of this thesis, house crickets currently could not be defatted due to legislative restrictions of insect use. Chinma et al. (2007) used soy flour with particle size of 100 μm, which can be used as a secondary reference.

Even though meat replacement would be the most logical use of house crickets, how does the particle size correspond to other food applications? Zucco et al. (2011) found that supplementing fine pulse flours greatly increased hardness and decreased spread of cookies, which are usually desirable features. Coarse flours on the other hand marginally reduced both parameters. From the flours used by Zucco et al. (2011), coarse green lentil flour was the only one even close to the particle size class of the cricket flours produced in this study, with mean particle size of 189.74 ± 5.33 μm. Besides, the cookies supplemented with coarse green lentil flour had unacceptable structure and were sticky to handle. Although the unacceptable structure and stickiness is probably caused also by other factors, like chemical composition, or altered water absorption capacity suggested by the authors, more fine-grained house cricket flours could possibly work better in cookies.

Dhen et al. (2016) also noted that the addition of finest soy flours produced gluten-free sponge cakes with higher specific volume compared to cakes with coarser flour added, again desirable trait. This was so regardless of the coarsest soy flour increasing the viscosity of the batters more, which could aid in incorporating and retaining more air and thus increasing the volume. Dhen et al. (2016) again used much finer flours with finest having mean particle size of < 132 μm, intermediate having 132-156 μm and coarses having > 156 μm, still much finer than the ones used in this thesis. De la Hera et al. (2013) stated as well that finer flours resulted in sponge and layer cakes with higher volume and lower firmness in comparison to coarser flours. Gómez et al. (2010) showed that flours with smaller particle size produced cakes with larger volume and better texture.

Particle size distribution affects also to the extractability of protein. Russin et al. (2017) studied soy flours with particle sizes of 89,5 ± 1,1; 184,2 ± 1,6 and 223,4 ± 6,4 μm, of which the coarsest flour is somewhat equivalent to the samples with smallest particle sizes used in this thesis (210 and 232 μm

for Oven-dried and Freeze-dried 0,5 mm samples, respectively). Russin et al. (2017) could increase the protein recovery of the material significantly ($p < 0,05$) from 40 to 52 % by decreasing the particle size of the flour. According to Vishwanathan et al. (2011) as well, smaller particle size increases protein extractability. Vishwanathan used a much broader particle size range from 710 μm all the way down to 75 μm and less. Maximum protein recovery was 97 % with fine soy flour fractions with particle size of < 75 μm. Coarse fractions with particle size of 355-710 μm had only 41 % protein recovery. With coarser fractions, secondary grinding improved the overall protein recovery by 6,8 %. Secondary grinding could therefore be good improvement for insect flour processing as well. These findings could have relevance in the future in house cricket protein isolate development, given the food-legislation allows separation of insect fractions.

Although it is clear that the optimal particle size distribution of the house cricket flour depends on what food application it would be used for, based on the examples presented above smaller mean particle size can be considered a desirable trait when the application is not yet known. Furthermore, the samples of this study seem to have larger particle size compared to the examples from the literature and thus too small particle size does not seem to be a problem in house cricket flours. Therefore, the optimal sample in terms of particle size distribution would be the one with smallest mean and median particle size, i.e. Oven-dried 0,5 mm sample followed by Freeze-dried 0,5 mm sample.

However, as mentioned in section 2.2.3., oven-dried samples also caused smearing in the mill-sieve and thus the relationship between the drying method used and the degree of smearing during milling could be interesting topic for further research.

Why did the Oven-dried samples have smaller mean and median particle size compared to Freeze-dried samples? Although the particle sizes varied significantly ($p < 0,05$) further studies are needed to tell, whether the drying method would have relevant effect on the particle size of the flour. Along with the different drying application, the difference could be explained by different water content, water activity, chemical composition, heterogeneity of the material, human error or various other factors. The oven-dried samples smeared during milling and freeze-dried did not, so the fat and its reactions during drying method could have a role in the final particle size of the flour.

4.3. Limitations of the study

One of the challenges in this study was due to novelty of insects as food and thus lack of scientific research. Therefore a lot of assumptions were made and compensatory studies were used to give support to the study. For instance during milling, similar rotating speed was used than with oat flours at the University before, because the fat profile was similar in between the materials. However, in order to get more thorough results, the rotating speed should be optimized specifically for house cricket material.

In this study 3 separate batches were analysed once for each result and therefore repetitions were not used. With the usage of repetitions, statistical quality of the results could be improved. Uncertainty can be can be caused by variability of house cricket size, insufficient experimental design, human error and other factors. In the experimental design, there are endless outside factors to control, which could increase the quality of the results, such as drying the same amount of samples simultaneously in the dryers which could affect the drying time.

Furthermore, soy flour and other materials were used as a reference for particle size distribution in this study due to lack of studies on house cricket material. However though the house cricket flour may be used in similar ways to fat containing and de-fatted soy flour, i.e. as meat replacement, the technological properties might differ and thus studies on house cricket flour should be used as a reference in the future.

In addition, it should be noted that conclusions made in this study are case-specific. The results would differ based on the material, equipment and parameters used. The equipment used in this study were those available at the university and were not industrial scale equipment. Improved results could be obtained by doing the experimental part of the study in production facilities.

Although the water content did not seem to increase much due to the decontamination process, according to the water content results of undried samples obtained in this study compared to water content of fresh house cricket from Finke (2002), the decontamination process could cause heterogeneity in the sample material water content. This in turn could have affected the results of this study. Additionally, water-soluble nutrients could leak out from the crickets during boiling and deteriorate the excellent micronutrient composition of house crickets expressed in table 1. Therefore, other methods such as steam or microwave-decontamination should also be considered. One way that the drying process of the cricket material could be optimized further would be to crush the cricket material in to a pulp before drying them as Arsiwalla and Aarts (2015) suggested. This way drying

time and energy could be reduced due to larger surface area to volume ratio. In this study, other drying parameters, such as temperature and pressure were kept constant. More complex study should be done in the future with multiple variable parameters and analysis on their impact on the end product. Furthermore, availability of equipment was a restrictive factor in this study and studies should be done at a larger scale, resembling more of industrial production facilities.

4.4. Suggestions for future improvements

Following are discussion on different areas of improvement for the future. The crickets used in this thesis were slaughtered by freezing straight to freeze-storage temperature at the farm, which is most common and humane way to slaughter crickets, since they are exothermic (van Huis and Tomberlin 2017). At the University facilities they then had to be decontaminated by boiling and be frozen back to freeze-storage temperatures. To make the supply chain more time- and energy-efficient, the crickets could be decontaminated before freezing to storage temperature. In this scenario, the crickets would first be slaughtered with careful expert consultation to ensure animal welfare. After being diagnosed dead, they would be decontaminated immediately and not frozen to freeze storage temperatures. After decontamination they would be frozen, transported and eventually processed. Obviously, if decontamination would be done at the farm, extra care should be taken to avoid contamination.

Although particle size distribution is an important quality factor, functional properties, such as emulsion capacity and stability, bulk density, water and oil absorption capacity, water solubility as well as foam capacity and stability of house cricket flour should be studied. For instance, Akpossan et al. (2015) showed that defatting *Imbrasia oyemensis* flours led to significant ($p < 0,05$) reduction of emulsion capacity and stability, but also significant ($p < 0,05$) increase in dispersibility, bulk density, water absorption capacity, water solubility index, oil absorption capacity and foamability. Their conclusion was that although defatting had a significant effect on the functional different properties of the insect flour, both full-fat and defatted flours would be suitable for food applications in terms of their functional properties (Akpossan et al. 2015).

5. Conclusion

In this study, an optimal product is the one within the water content and –activity safety limits with minimal amount of drying time as well as smallest average particle size. As a conclusion, optimal measured drying time at T=70 °C for oven-drying was 5 hours. For Freeze-drying at T_p=25 °C, T_c=-87 °C, p=1 mbar was 6 hours. Of the optimally dried samples, Oven-dried sample milled with 0,5 mm sieve size had the significantly lowest mean and median particle size followed by Freeze-dried sample milled with 0,5 mm sieve size. The 5-hour Oven-dried sample milled with 0,5 mm sieve size was thus the closest to the optimal product, because it had the smallest particle size from all the samples. Samples milled with 1 mm sieve size had mean and median particle size similar to that of the Reference samples obtained from the industry. Furthermore it can be concluded, that finer house cricket flour compared to reference flours can be milled with 0,5 mm sieve size and 1 mm sieve size with the particular centrifugal mill used yields a flour with similar coarse particle size than those used in the industry today. However, Oven-dried samples caused smearing during milling and thus the relationship between drying method and smearing should be studied further. All of the milled samples showed narrower particle size distribution compared to the Reference samples, which indicates more homogenous flour, a desirable trait as long as a specific food application is not known. This thesis gave a reference to future house cricket processing projects and produced public scientific information to theme, most commonly studied privately within the R&D departments of food companies. The findings of this study should be used to study the opportunities to use house cricket flour further to produce safe, sustainable and healthy food products to consumers.

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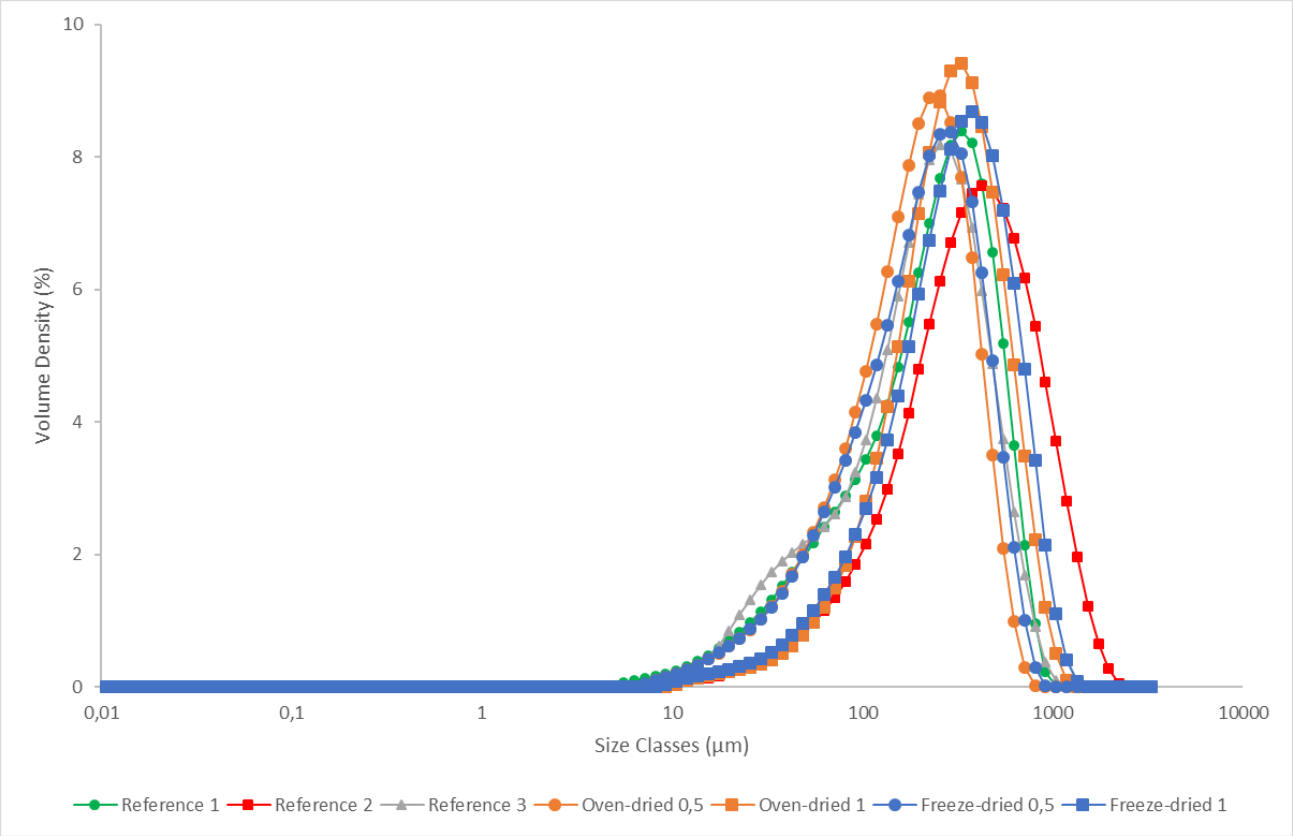
7. Appendices

Appendix 1. Particle size distributions of all measured samples.



Appendix 2. Formulas.

Formula for calculating Obscuration

Obscuration can be calculated using formula (1) from Malvern Instruments (2013), where L_s is the light intensity measured in the central detector when a sample is present in the cell and L_b is the same but with clean dispersant:

$$Ob = 1 - \frac{L_s}{L_b} \qquad (1)$$

Formula for calculating Concentration

Concentration is calculated using Beer Lambert's law (4) from Malvern Instruments (2013), where I is the intensity of light at a distance b in the particle field of absorbance α, I_0 is the intensity of the light beam as it enters the particle field, I / I_0 is the relative transmission T of the beam (measured directly by the instrument). I_0 is the intensity of the laser beam measured at the receiver when no sample is present and I is the intensity with sample in the beam:

$$\frac{I}{I_0} = e^{-\alpha b} \quad (4)$$

Formula for calculating Span

Span was calculated with the formula (5), from Malvern Instruments (2013), using percentile readings Dx(10), Dx(50) and Dx(90):

$$Span = \frac{Dx(90) - Dx(10)}{Dx(50)} \quad (5)$$

Formula for calculating Uniformity

Uniformity was calculated with formula (6) from Malvern Instruments (2013), where $d(x,0.5)$ is the median size of the distribution and d_i and x_i are respectively the mean diameter of, and result in, size class i.

$$Uniformity = \frac{\sum X_i \, d(x,0{,}5) - d_i}{d(x,0{,}5) \sum X_i} \quad (6)$$

Formula for calculating Specific surface area

Specific surface area (SSA) was calculated with formula (7) from Malvern Instruments (2013), where V_i is the relative volume in class i with mean class diameter of d_i and p is the particle density:

$$SSA = \frac{^{6}\sum \frac{V_i}{d_i}}{p \sum V_i} \qquad (7)$$

Formula for calculating Volume Weighted Mean and Surface Weighted Mean

Volume- and Surface Weighted Means can be calculated with the generalized form of their equations, equation (8) from Horiba Instruments (2017), where the overbar in $\overline{D}$ designates an averaging process, $(p\text{-}q)p>q$ is the algebraic power of Dpq, D_i is the diameter of ith particle and Σ is the summation of Dip or Diq, representing all particles in the sample:

$$\overline{D}_{pq}^{(p-q)} = \frac{\sum D_i^p}{\sum D_i^q} \quad (8)$$